

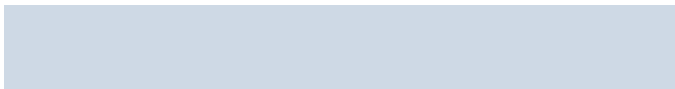
Antimicrobial peptides and device coating

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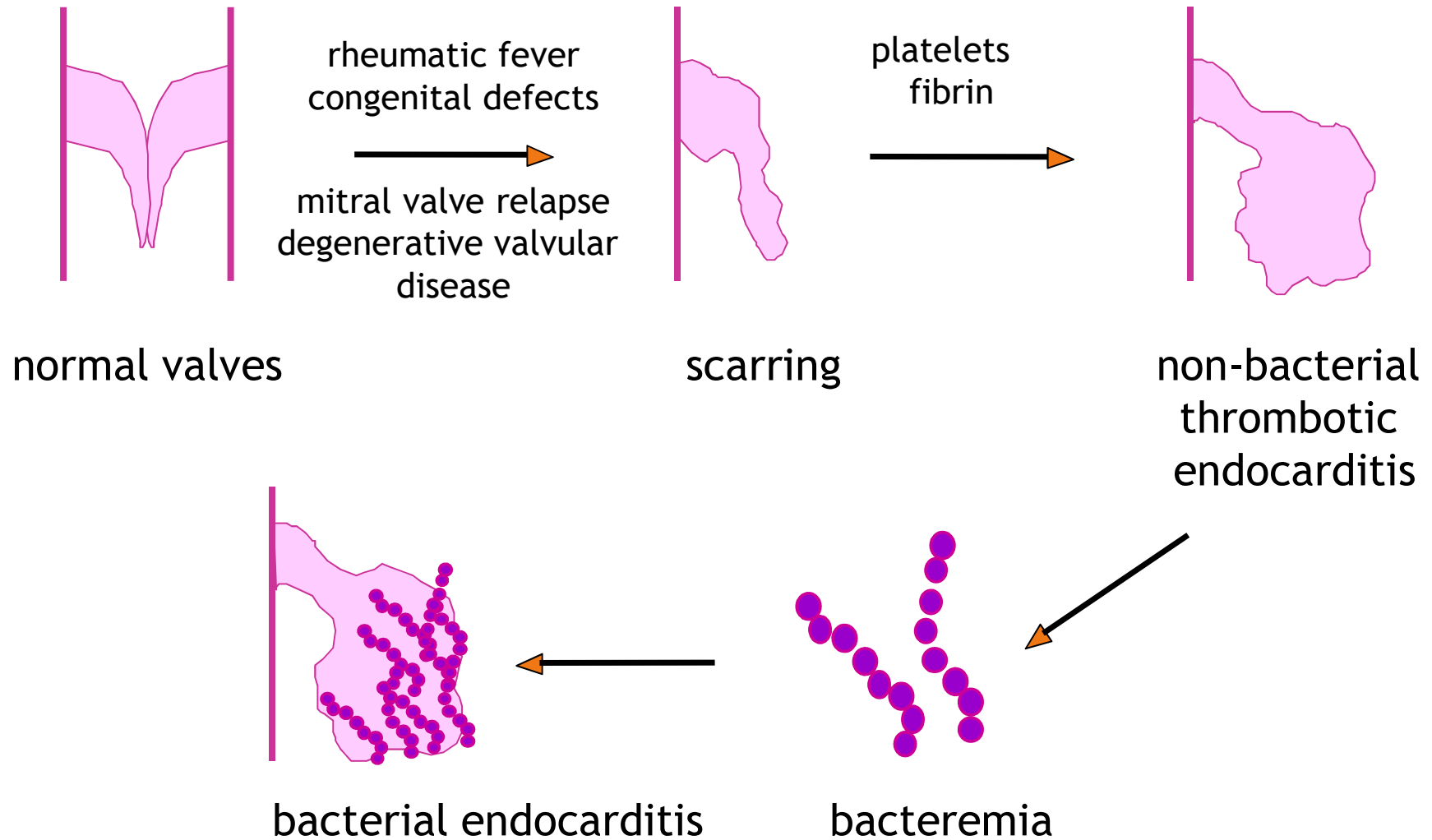


Outline

- Pathogenesis
- ES TE heart valves
- Synthetic Antimicrobial and Antibiofilm Peptides
- Antimicrobial supramolecular polymers
- Antimicrobial Photochemical internalization

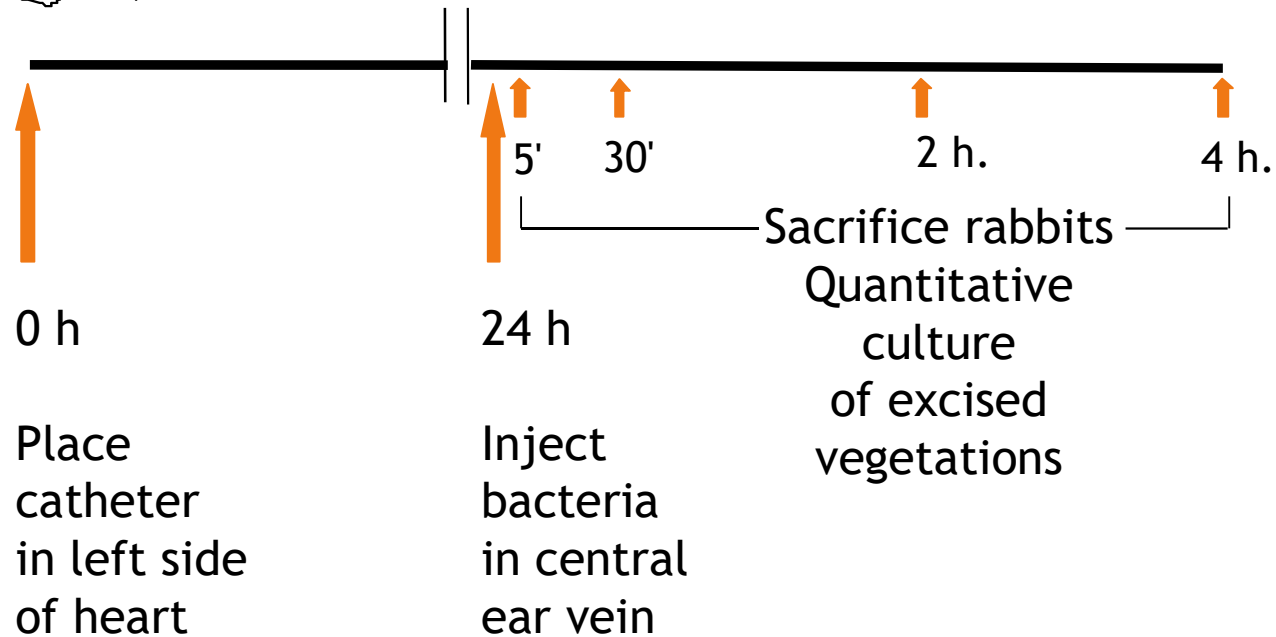


Native valve Streptococcal Endocarditis



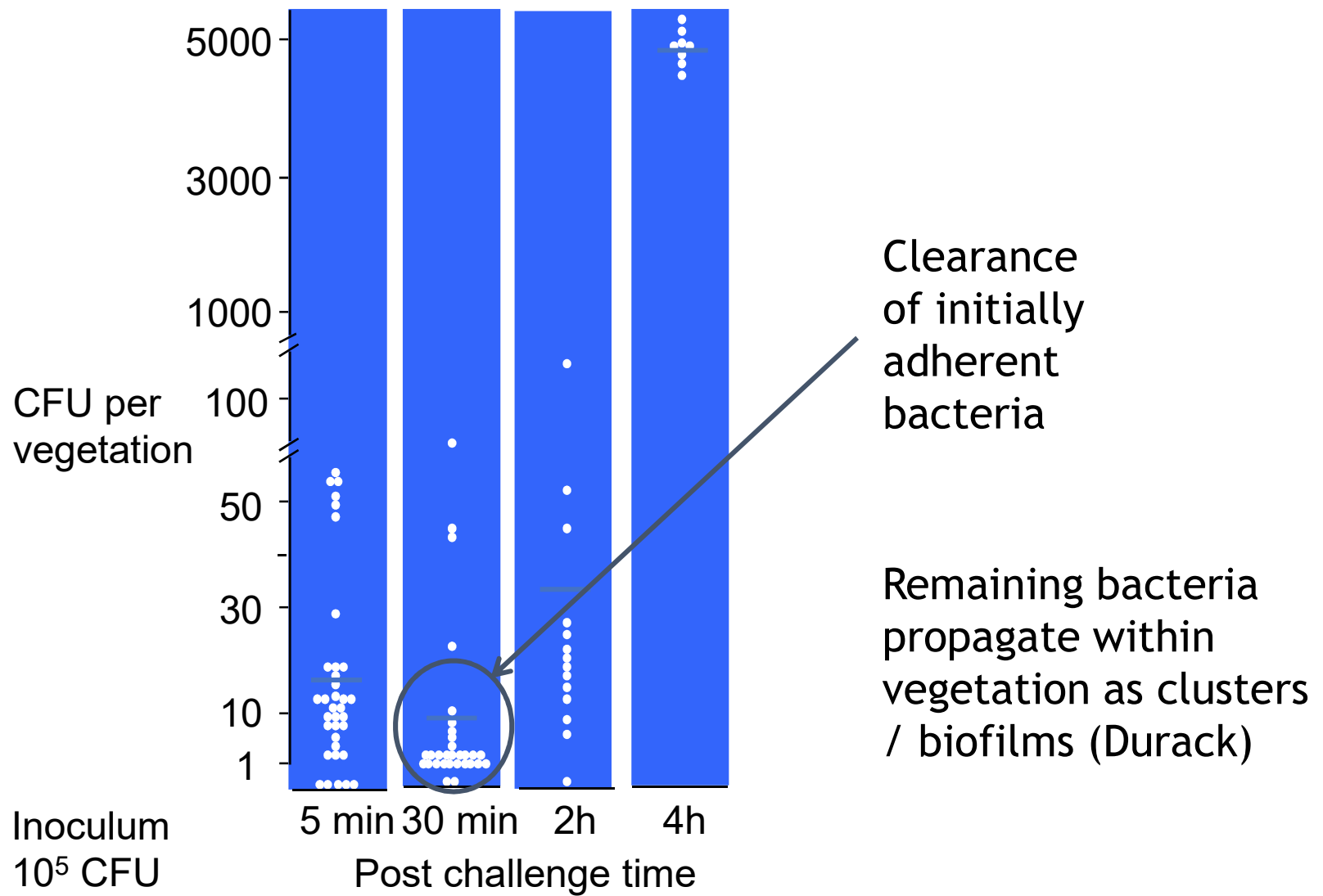


Rabbit Model of Experimental Infective Endocarditis

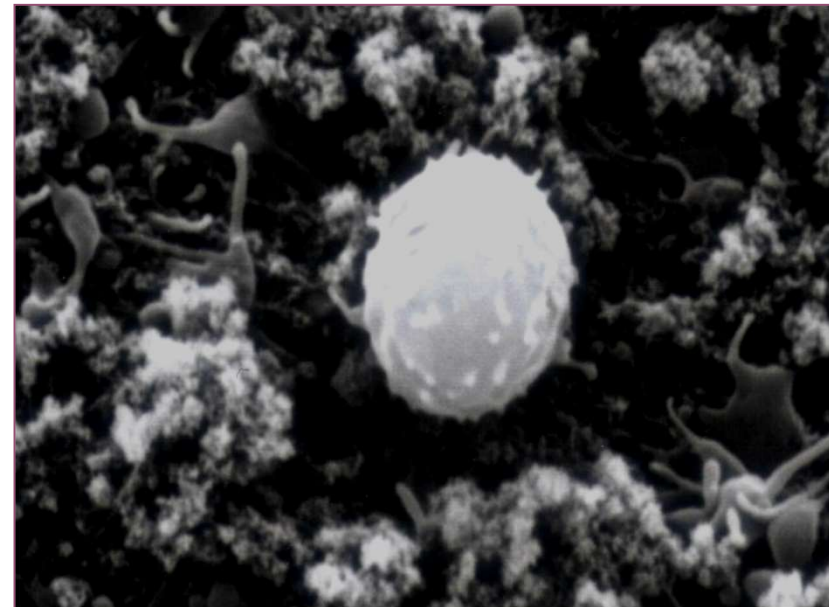
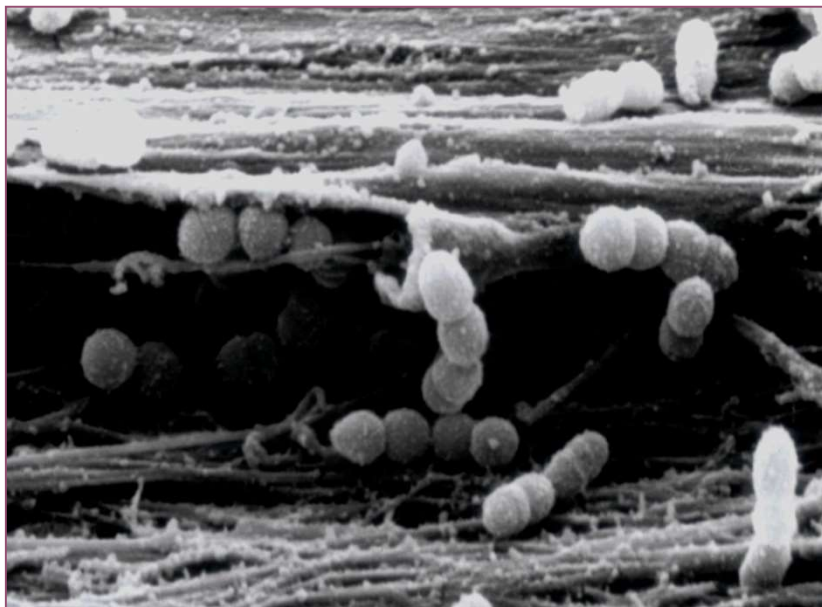
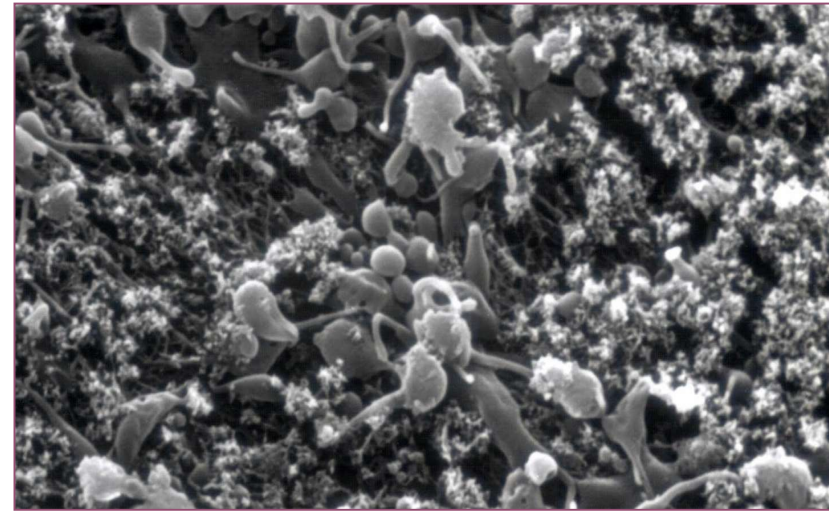




Initial Clearance of *Streptococcus oralis* from rabbit VGs



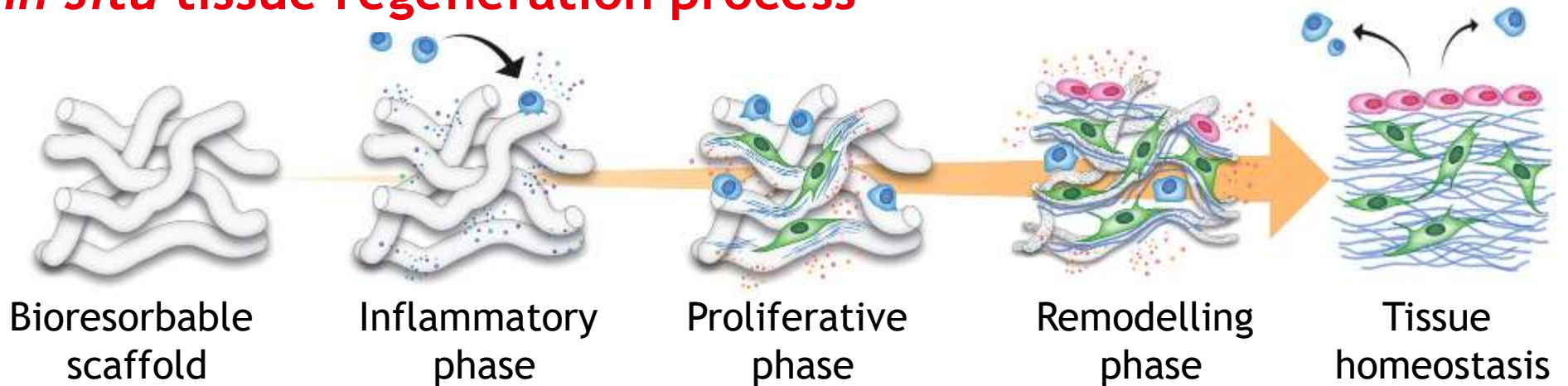
Viridans Streptococci Within Platelet-Fibrin Clots Are Shielded from Phagocytes



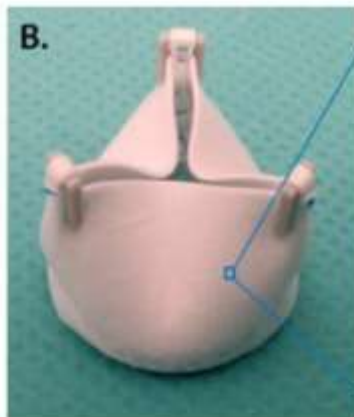
Biomaterial implants for *In situ* tissue engineering of heart valves



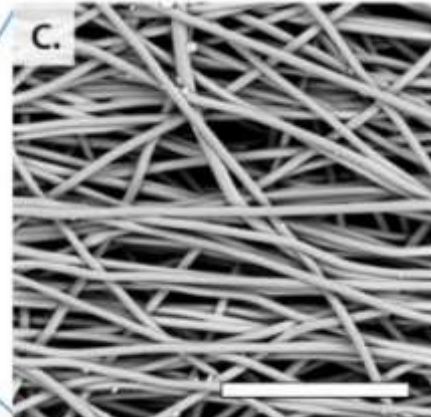
In situ tissue regeneration process



Heart valve



Electrospun scaffold



Heart valve in situ



Infection Risk ?



Incidence Native Valve IE Surprisingly Low

Vegetations in **2.4%** of 3404 autopsy specimen of hospitalized patients without IE

Daily low level transient bacteremia due to chewing candy, eating and tooth brushing: **7 - 51%**

Bacteria adhering to / embedded in vegetations are shielded from phagocytes: expected to cause IE!!

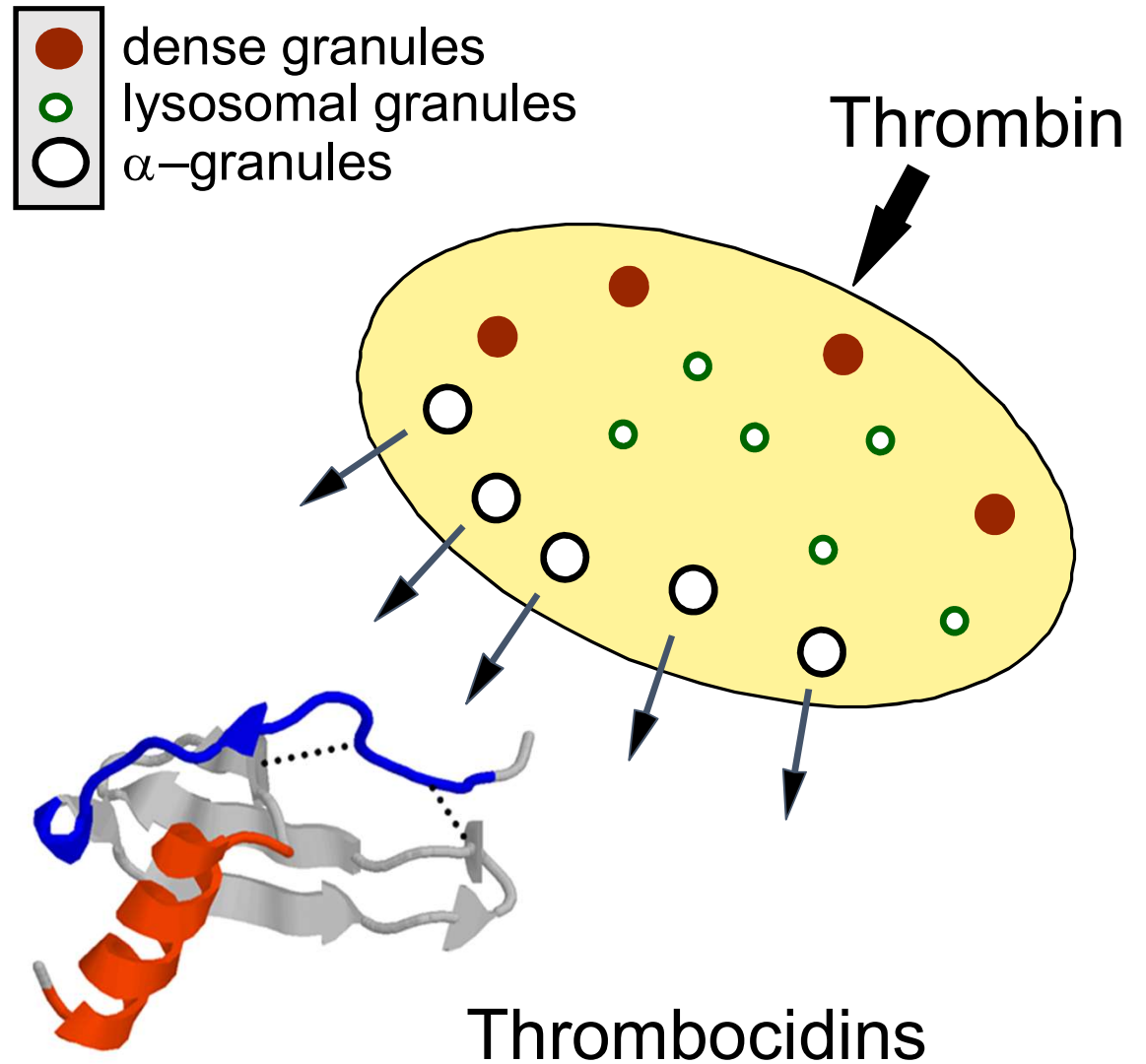
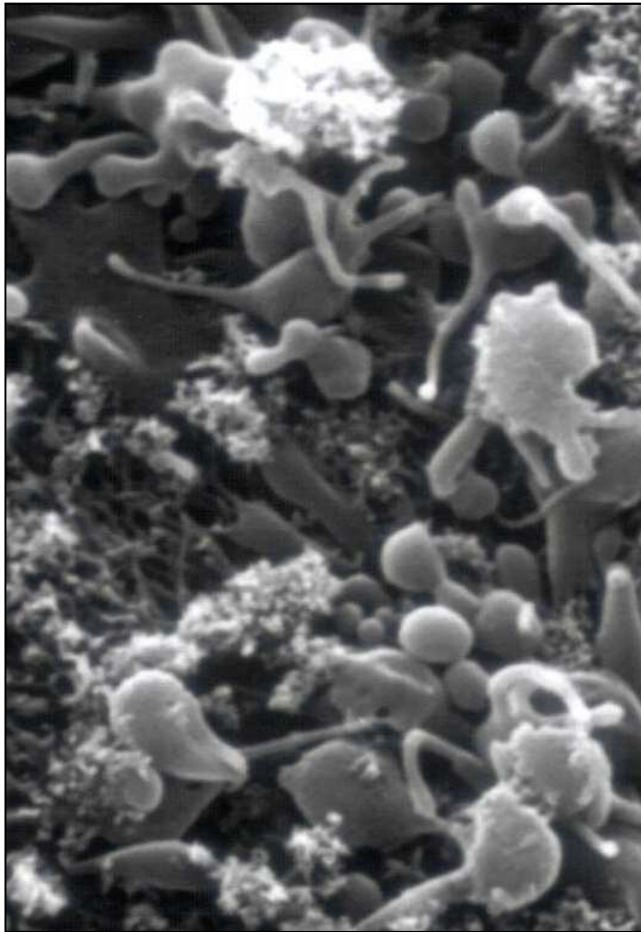
IE cases:

- General population: 0.7 - 6.8 per 100,000 per year
- Persons at risk for IE: 20 - 180 per 100.000 per year

➔ **PROTECTIVE MECHANISM: Thrombocidins / PMP**



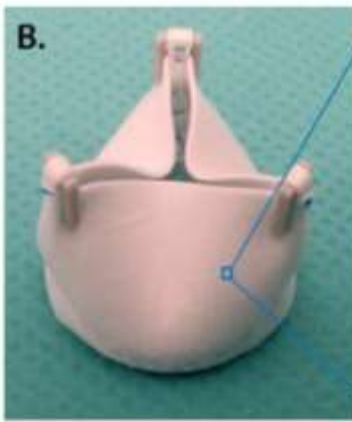
Human platelet Thrombocidins, cationic AMPs



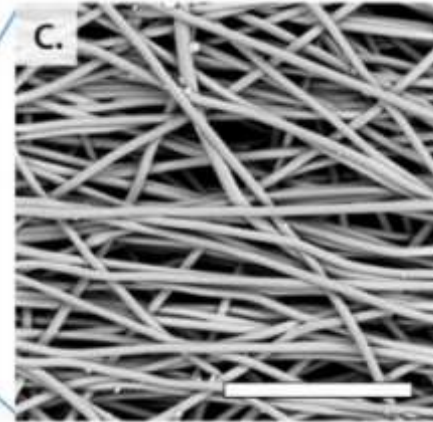


Can we provide similar protection to electrospun TE prosthetic valves?

Heart valve



Electrospun scaffold



Heart valve in situ



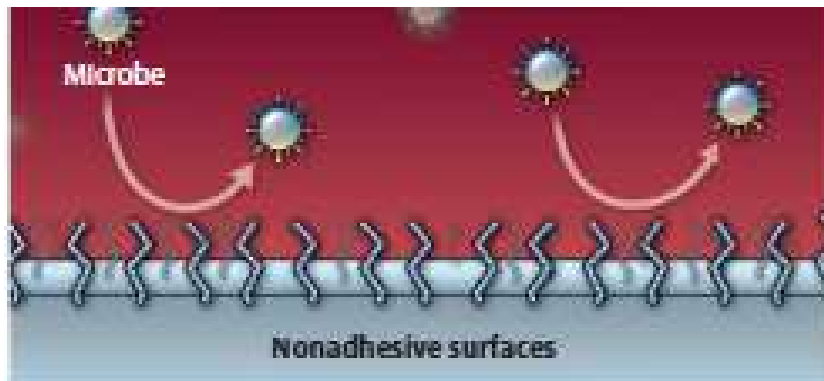
Requirements:

- Novel antimicrobials preventing biofilm formation
- No resistance development
- Manufacturing system

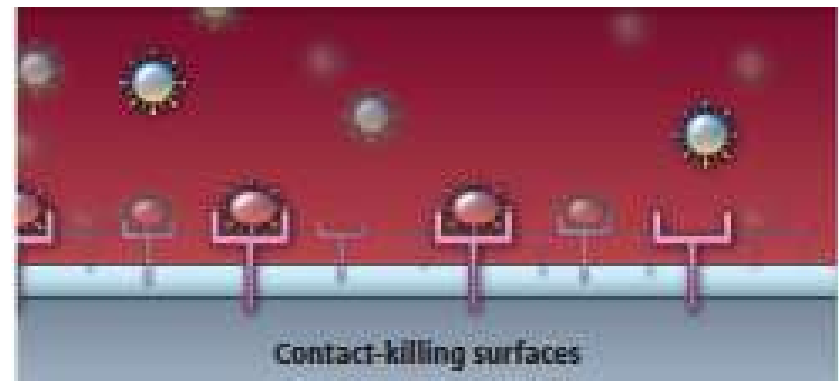


Antimicrobial surface designs

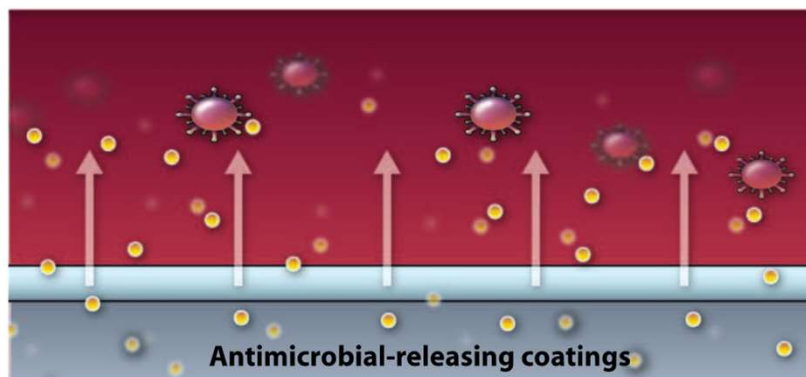
Anti-adherent



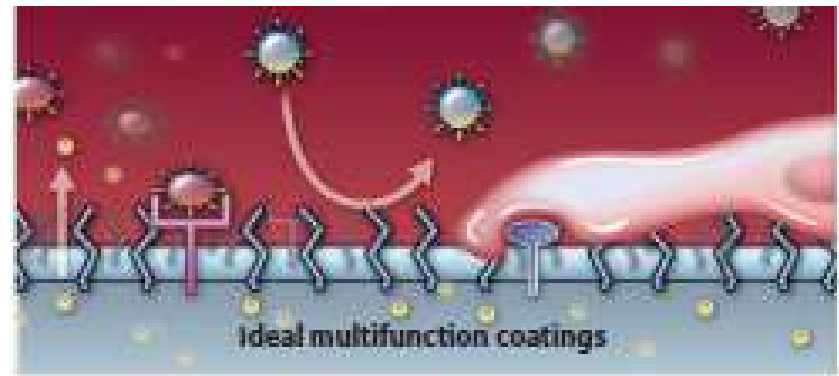
Contact killing



Release



Combinations



BALI, the Biofilm Alliance, EU FP7

Design Synthetic Antimicrobial and Anti-biofilm Peptides, SAAPs

- Inspired by human cathelicidin LL-37
- Inspired by human Thrombocidin-1

Develop therapeutic applications with SAAPs

- Controlled release coating for implant surfaces
- Ointment for skin wound infections



Martijn Riool



Anna de Breij

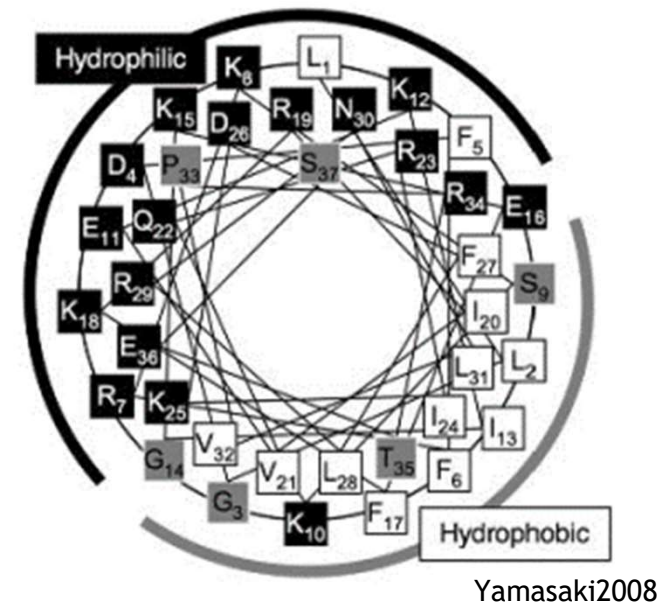
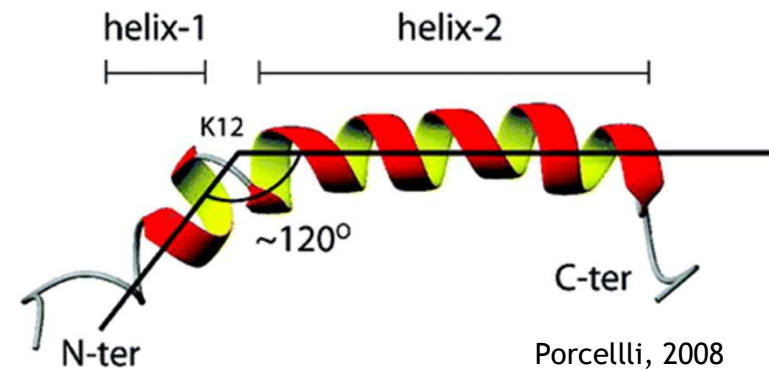


Leonie de Boer

Human cathelicidin LL-37, and Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs)



- Precursor hCAP18, produced by neutrophils and epithelial cells
- Amphipathic α -helical structure
- Derived peptides:
 - SAAPs (SAAP-148)
 - OP-145

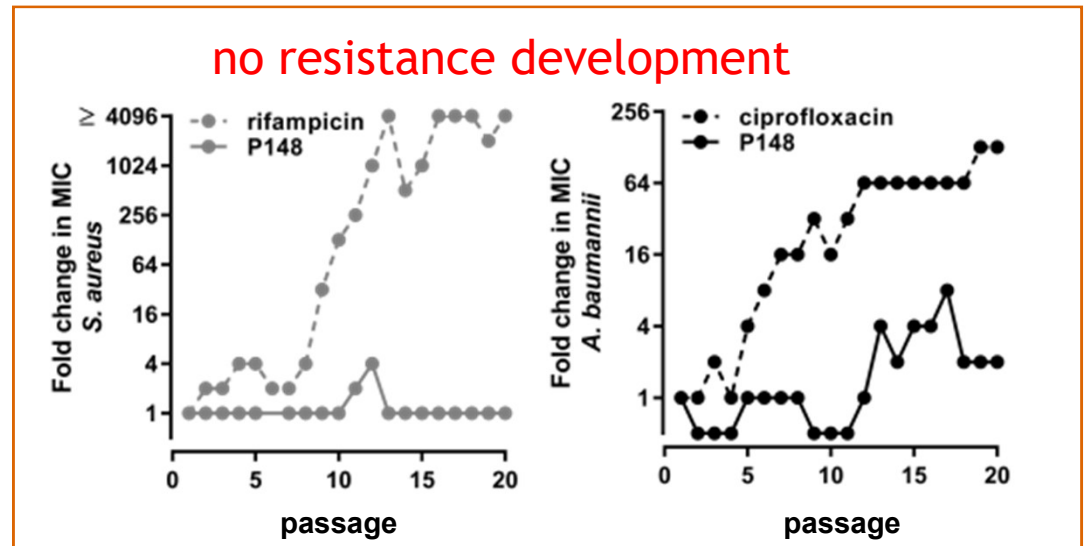
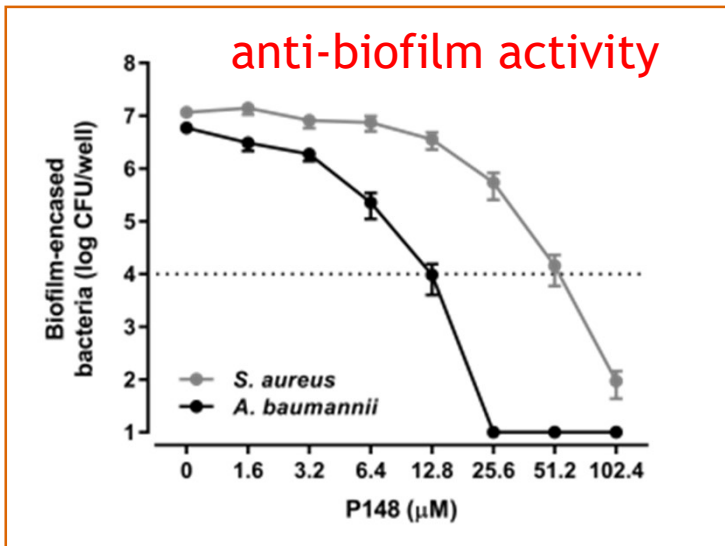


SAAP-148, the present lead compound



| Species | Strain | aminoglycosides | ansamycins | carbapenems | cephalosporins | fluoroquinolones | fusidanes | glycopeptides | glycylcyclines | lincosamides | lipopeptides | macrolides | monoxycarbolic acid | oxazolidinones | penicillins | polymyxins | sulfonamides | tetracyclins | LC99.9 P148 (μM) | |
|----------------------|----------|-----------------|------------|-------------|----------------|------------------|-----------|---------------|----------------|--------------|--------------|------------|---------------------|----------------|-------------|------------|--------------|--------------|-------------------------------|------------|
| | | | | | | | | | | | | | | | | | | | PBS | 50% plasma |
| <i>E. faecium</i> | LUH15122 | | | | | | | | | | | | | | | | | | 1.6 | 6.4 |
| <i>S. aureus</i> | LUH14616 | | | | | | | | | | | | | | | | | | 1.6 | 3.2 |
| <i>K. pneumoniae</i> | LUH8995 | | | | | | | | | | | | | | | | | | 0.4 | 3.2 |
| <i>A. baumannii</i> | RUH875 | | | | | | | | | | | | | | | | | | 0.8 | 1.6 |
| <i>P. aeruginosa</i> | LUH15103 | | | | | | | | | | | | | | | | | | 6.4 | 12.8 |
| <i>E. cloacae</i> | LUH15114 | | | | | | | | | | | | | | | | | | 12.8 | 3.2 |
| <i>E. coli</i> | LUH15117 | | | | | | | | | | | | | | | | | | 0.8 | 6.4 |

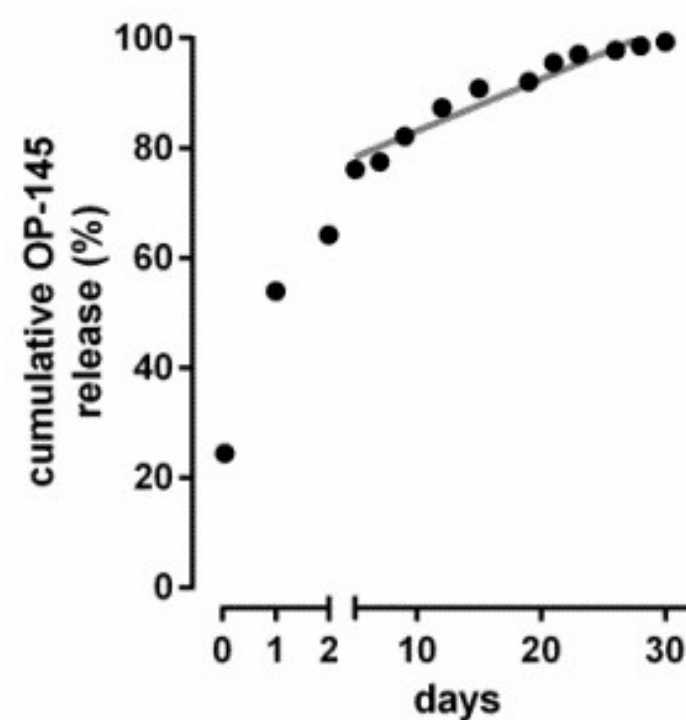
Broad range
Activity;
ESKAPE panel



OP-145 Polymer Lipid Encapsulation Matrix (PLEX) for titanium implants



Composed of PLGA / DPPC / DSPC / Cholesterol / **OP-145** (10 wt%)



- Initial **burst release** 55% in the first 48h
- First order kinetic release (~1%) for 30 days
- OP-145 released from coating kills *S. aureus*



PLEX-OP-145 prevents rabbit humerus intramedullary nail infection

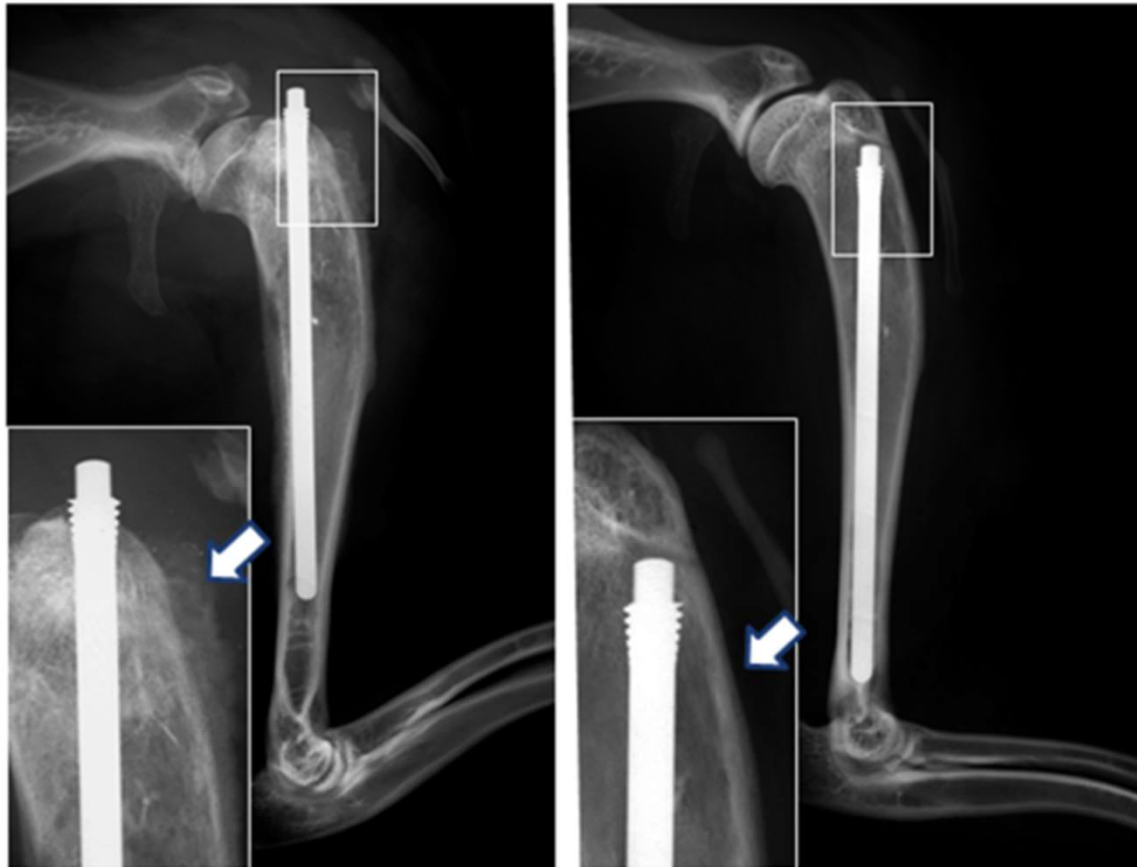
- Intramedullary (IM) nail infection model
 - New Zealand White rabbits
 - Right humerus
 - 6×10^4 CFU *S. aureus* JAR
 - TAN IM nail
 - No coat
 - PLEX-OP-145 coating
 - Evaluation at 28 days
 - Quantitative culture
 - Contact radiograph
 - Clinical parameters



 **AO Foundation**
Fintan Moriarty



PLEX-OP-145: contact radiographs

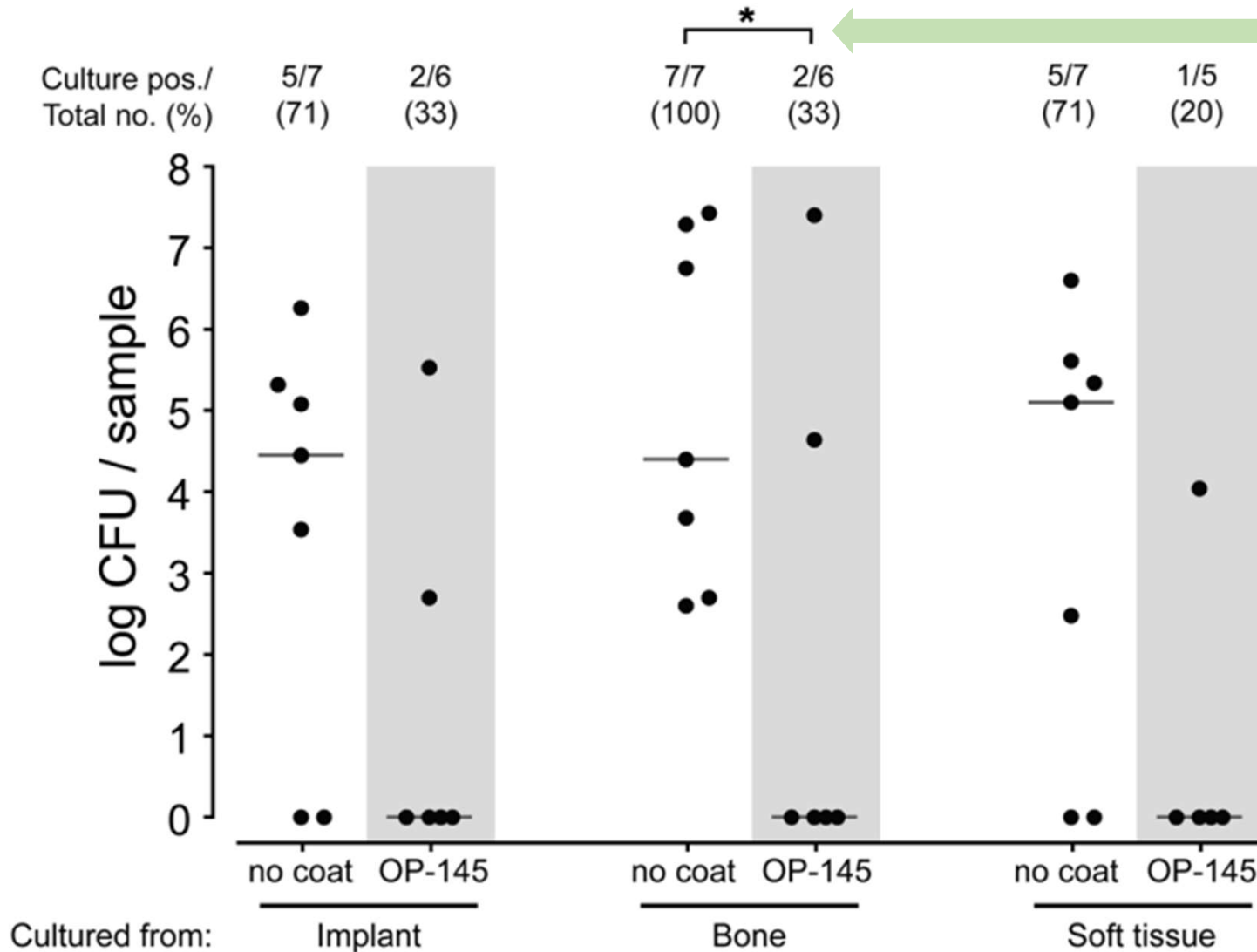


uncoated
Signs of infection

OP-145-coated
No signs of infection



PLEX-OP-145: quantitative culture

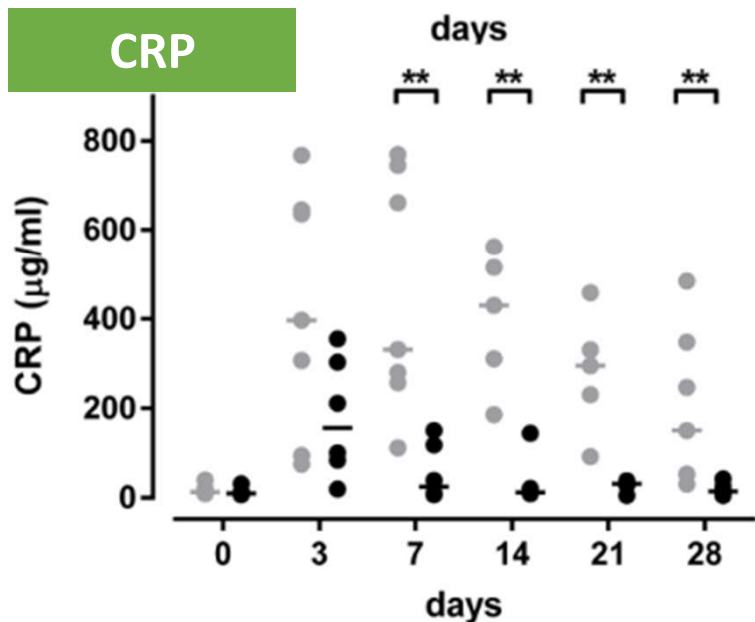
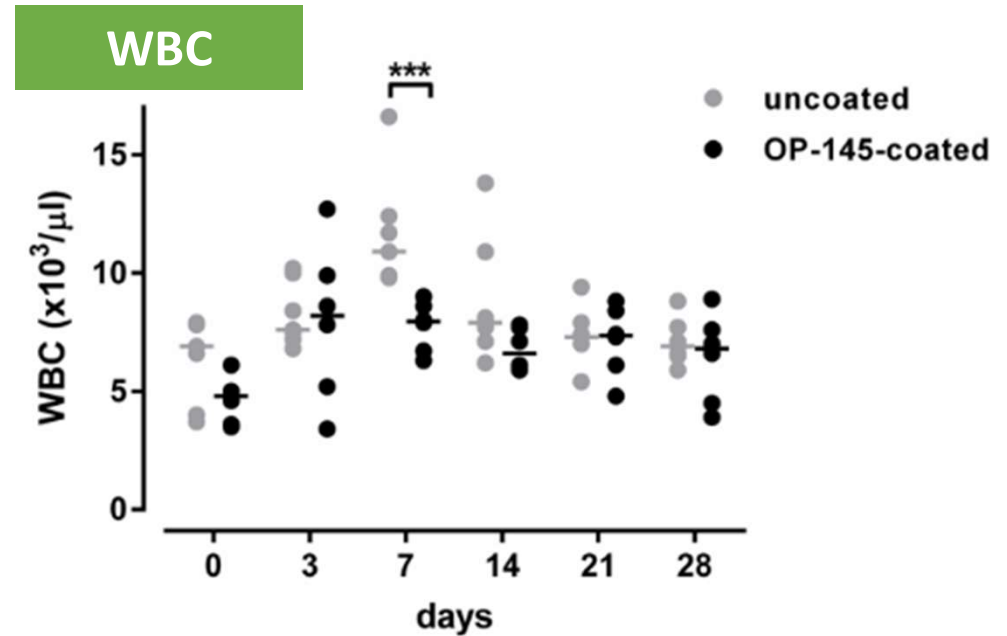
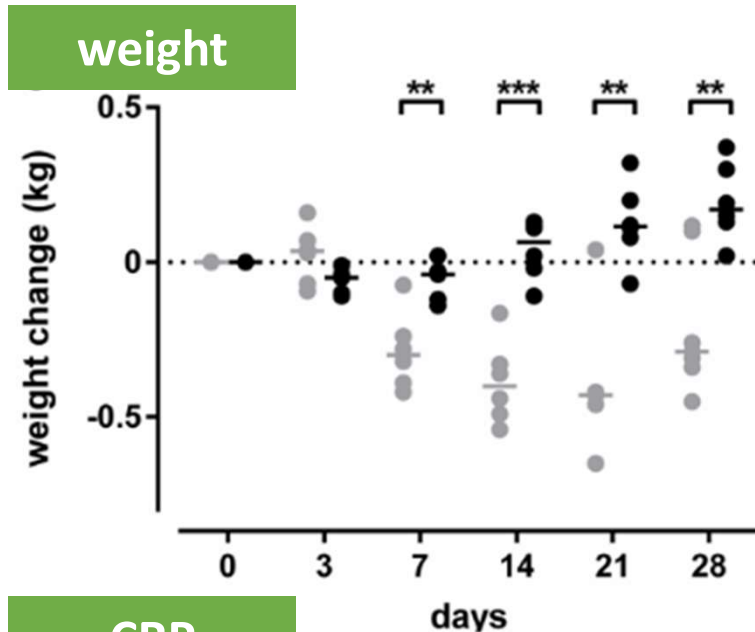


PLEX-OP-145 effective

Trend:
Reduction in numbers of CFU in all samples

50% of rabbits infection free

PLEX-OP-145: clinical parameters



Rabbits with uncoated implants:

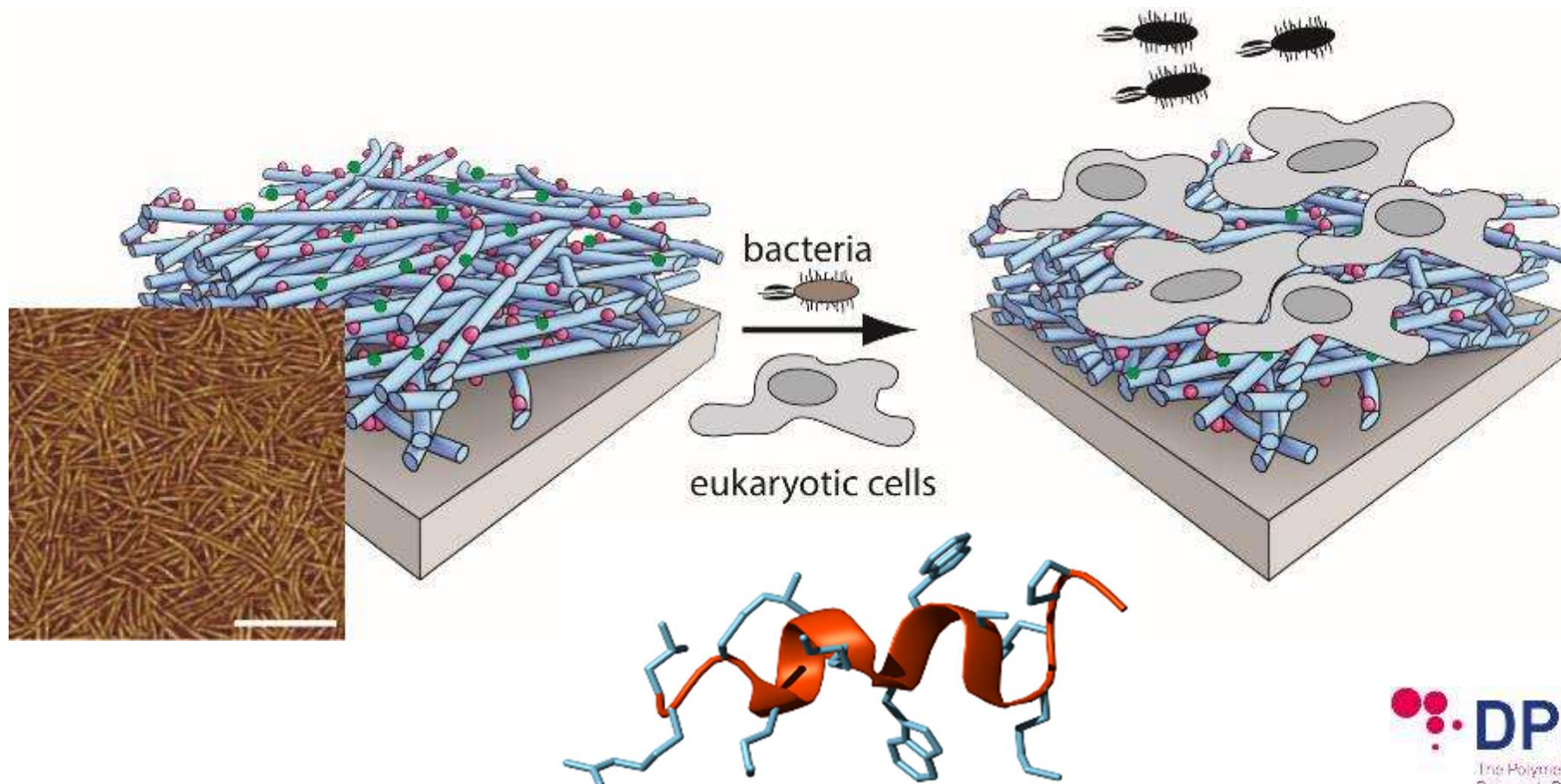
- more weight loss
- elevated WBC counts at 7d
- elevated CRP level $\geq 7\text{d}$

**→ Clinical signs of infection
PREVENTED WITH PLEX-OP-145**

Heart valves? SuperActive!

Supramolecular Biomaterials with Antimicrobial and Regenerative Activity

Development of multi-functional bioactive supramolecular materials with both antimicrobial and regenerative activity



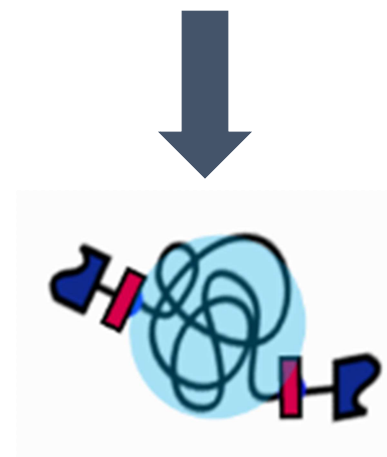
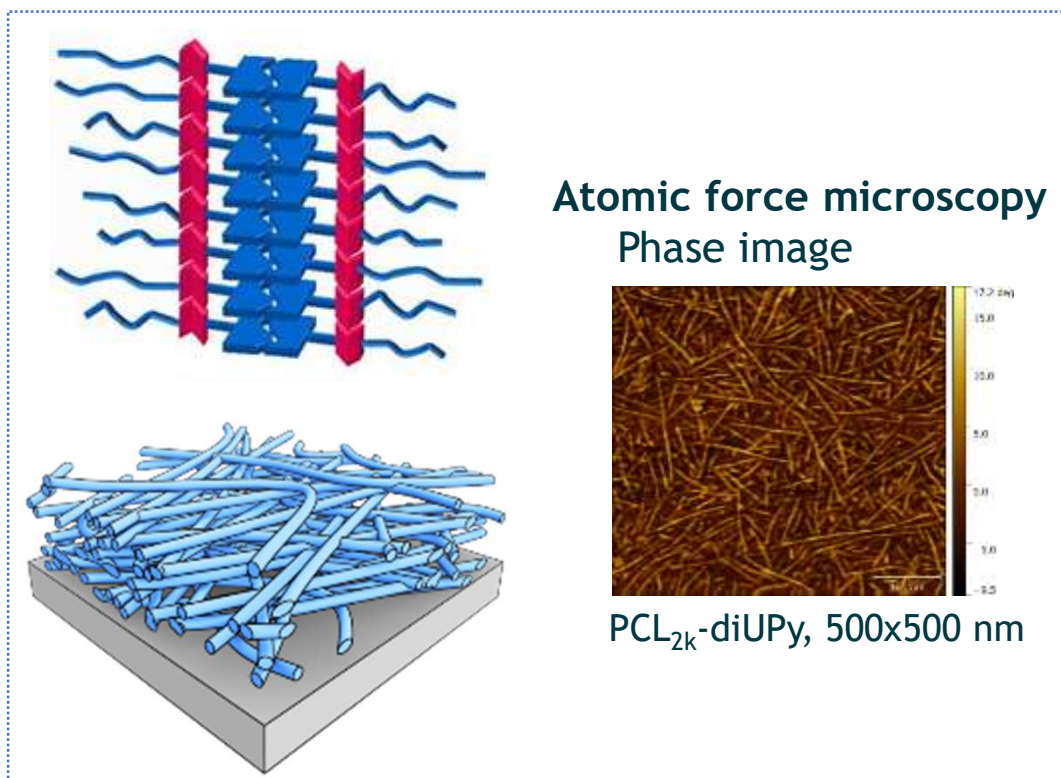
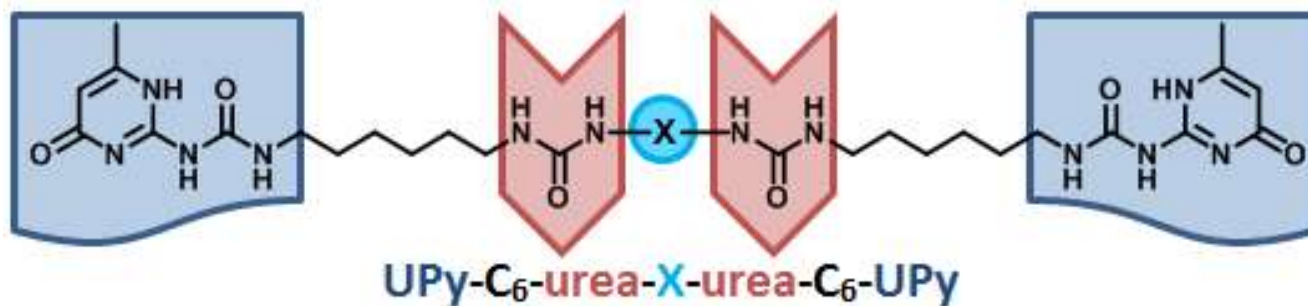
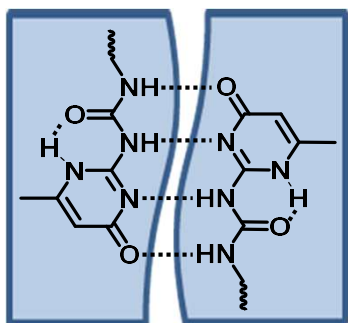
Antimicrobial activit

Novel AMPs **TC19** and **SAAP-148**

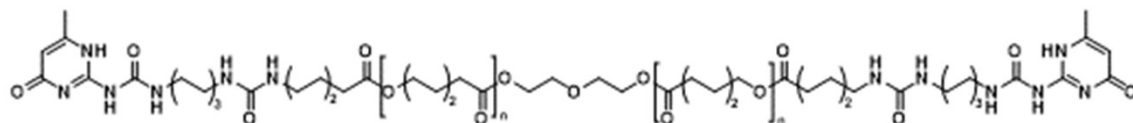
Supramolecular materials



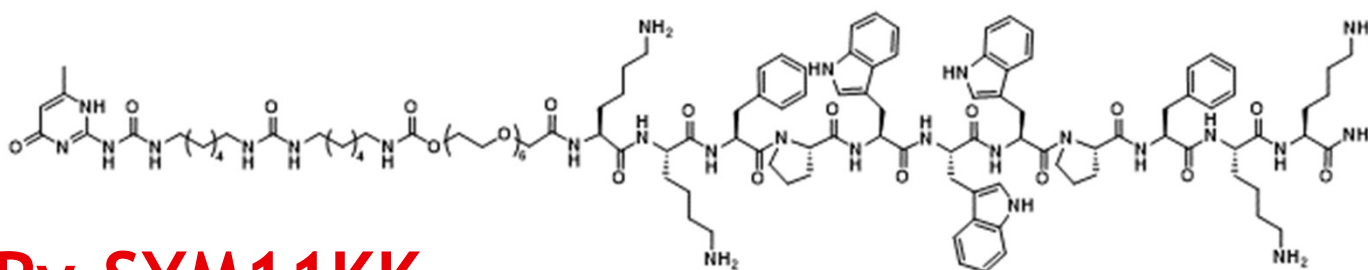
Ureido-pyrimidinone (UPy)



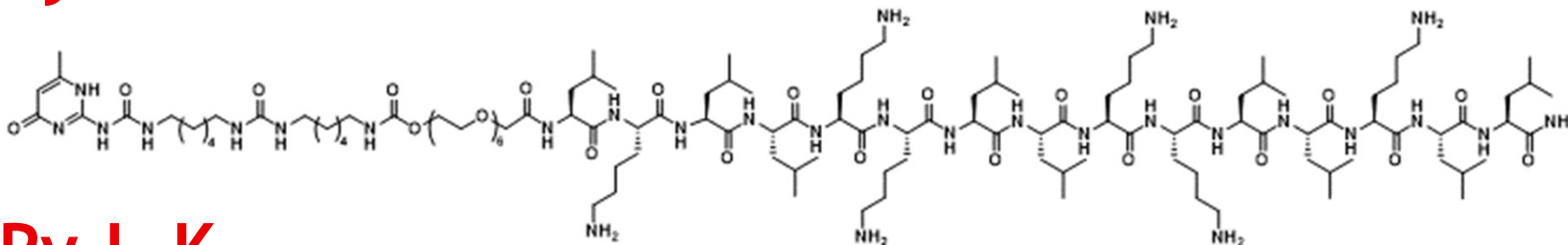
UPy-antimicrobial peptides (from literature)



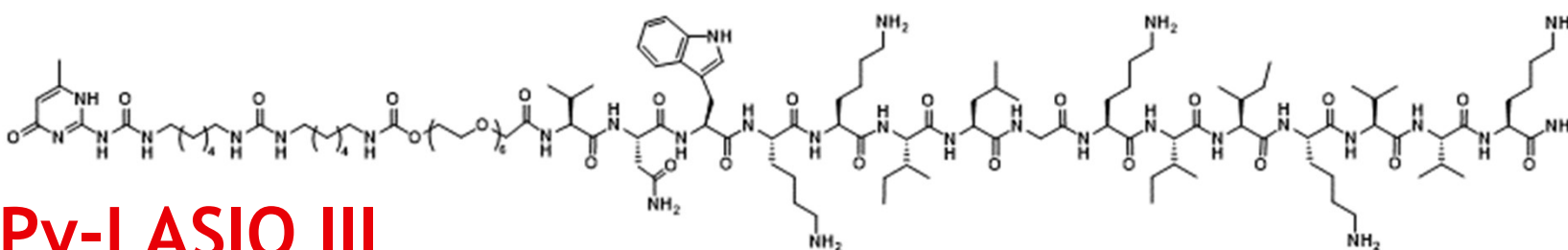
PCL_{2k}-diUPy



UPy-SYM11KK



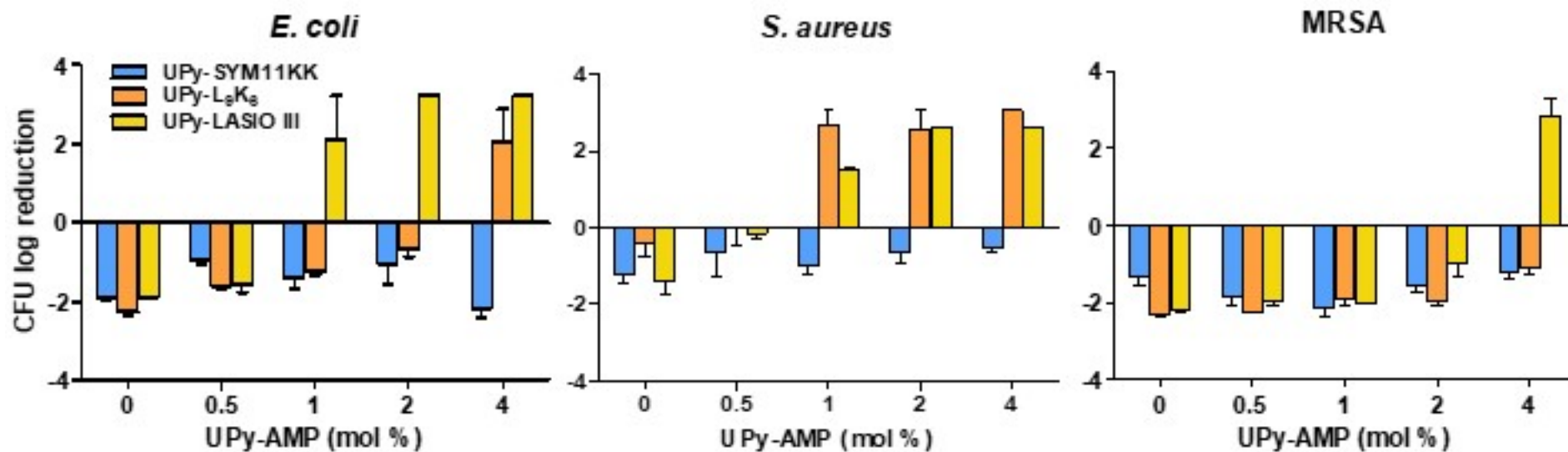
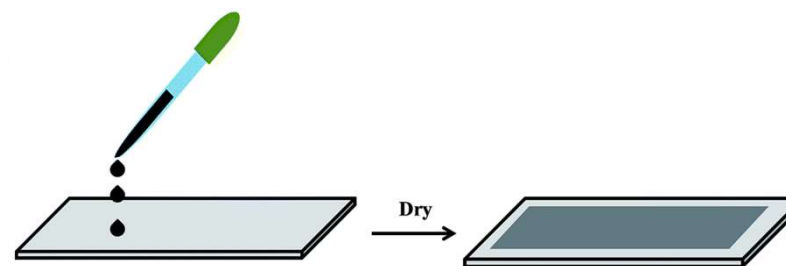
UPy-L₉K₆



UPy-LASIO III

Surface antimicrobial activity of UPy-AMP solid samples

Dropcasting of PCL_{2k}-diUPy films with 4 mol% (UPy-)AMPs



SuperActive plans

Development of multi-functional bioactive supramolecular materials with both antimicrobial and regenerative activity

Antimicrobial activity

- Novel AMPs **TC19** and **SAAP-148**



Regenerative activity

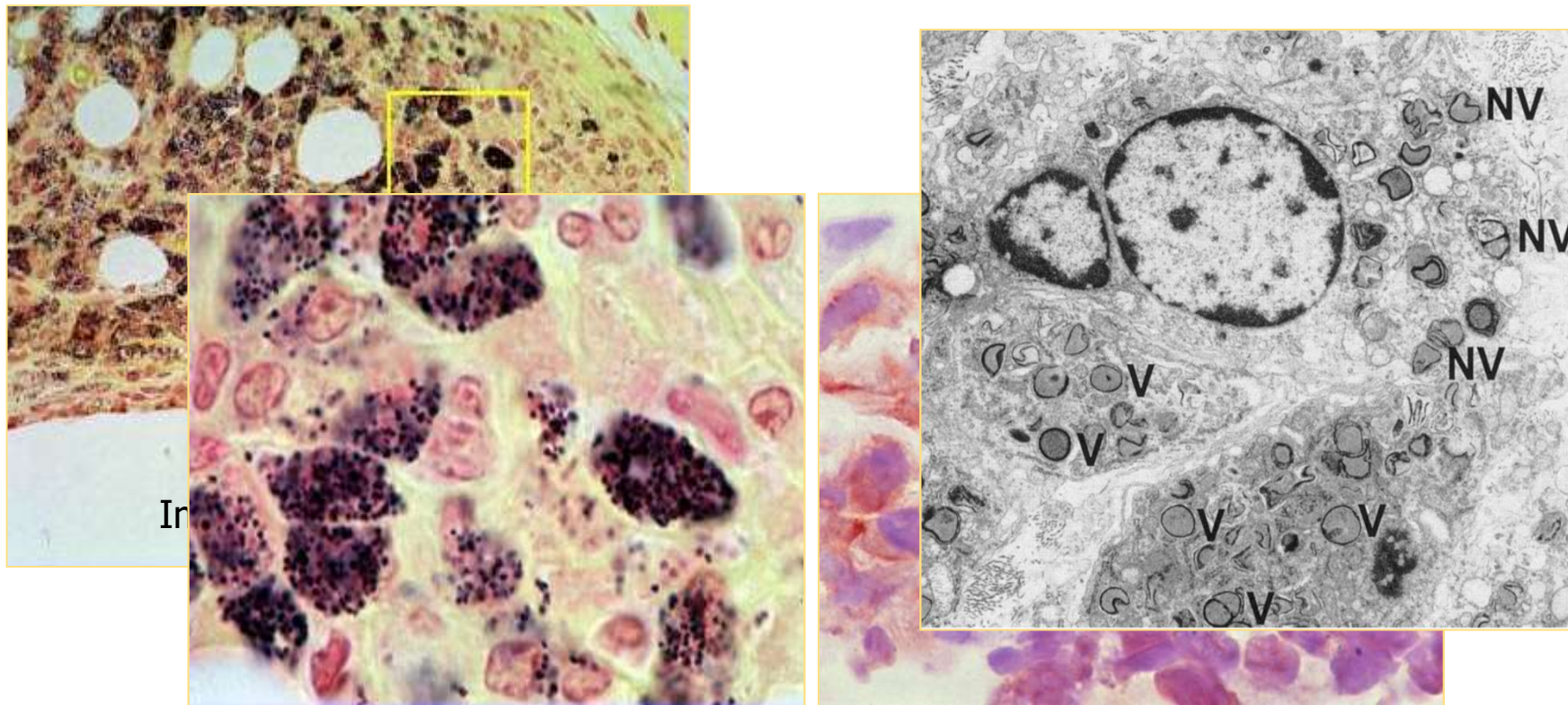
- Heparin-binding peptide (**HBP**)
- Cell-adhesive properties



And what about abscesses and intracellular bacteria.... ?



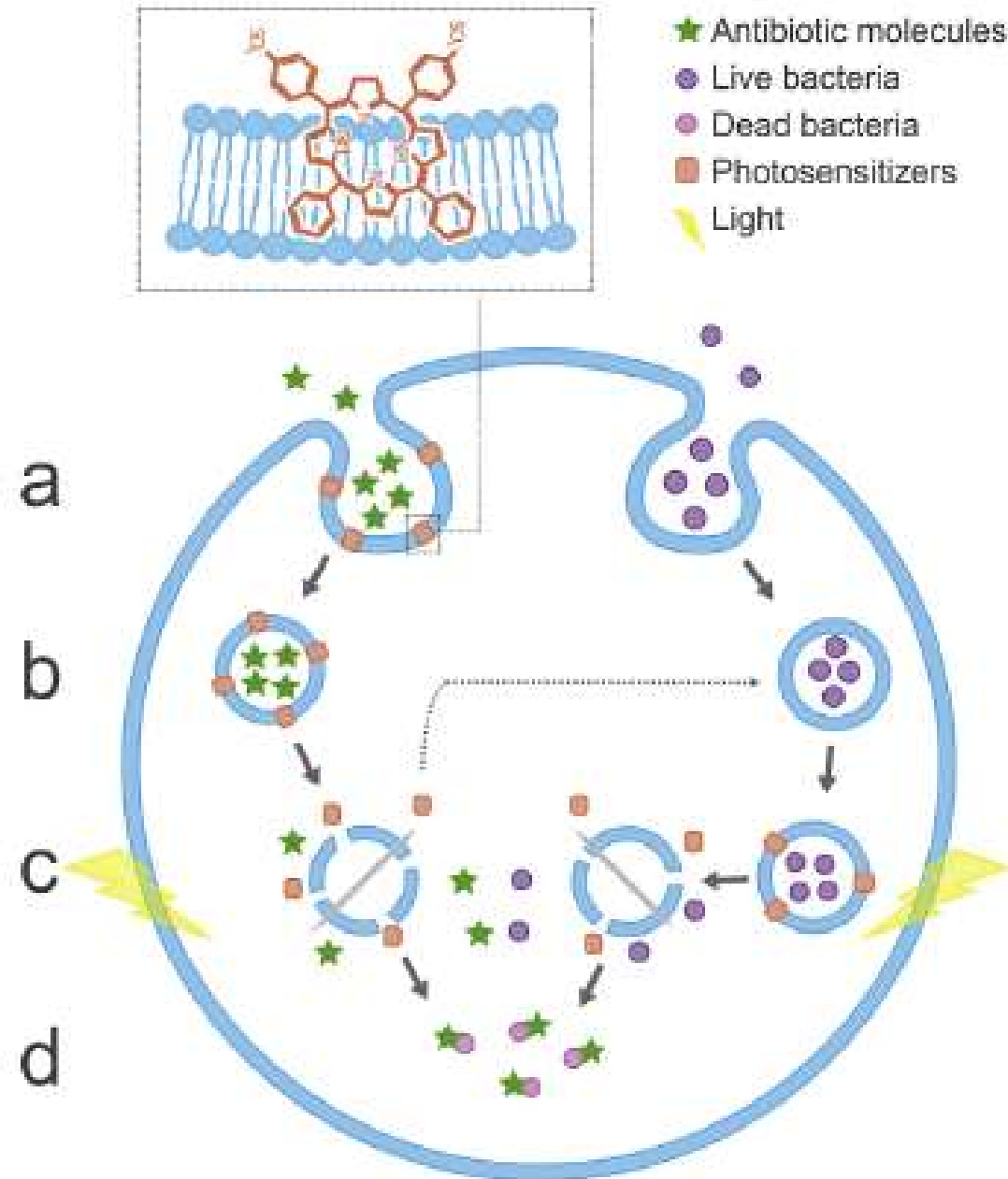
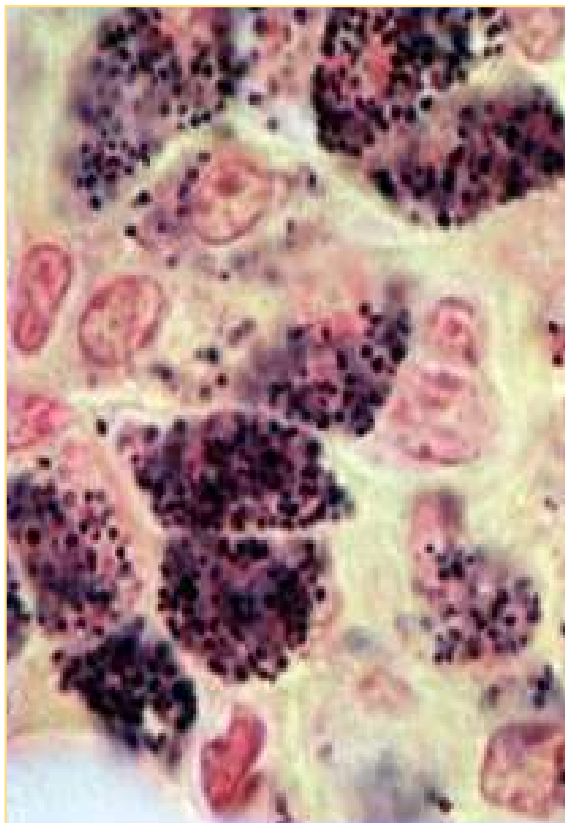
Intracellular *S. epidermidis* in mouse peri-implant subcutaneous tissue after 14 days



- Bacteria in tissue, within macrophages (F4/80)
- Mice **implant-associated sepsis** after 3 weeks
- Survival due to deranged cytokine responses

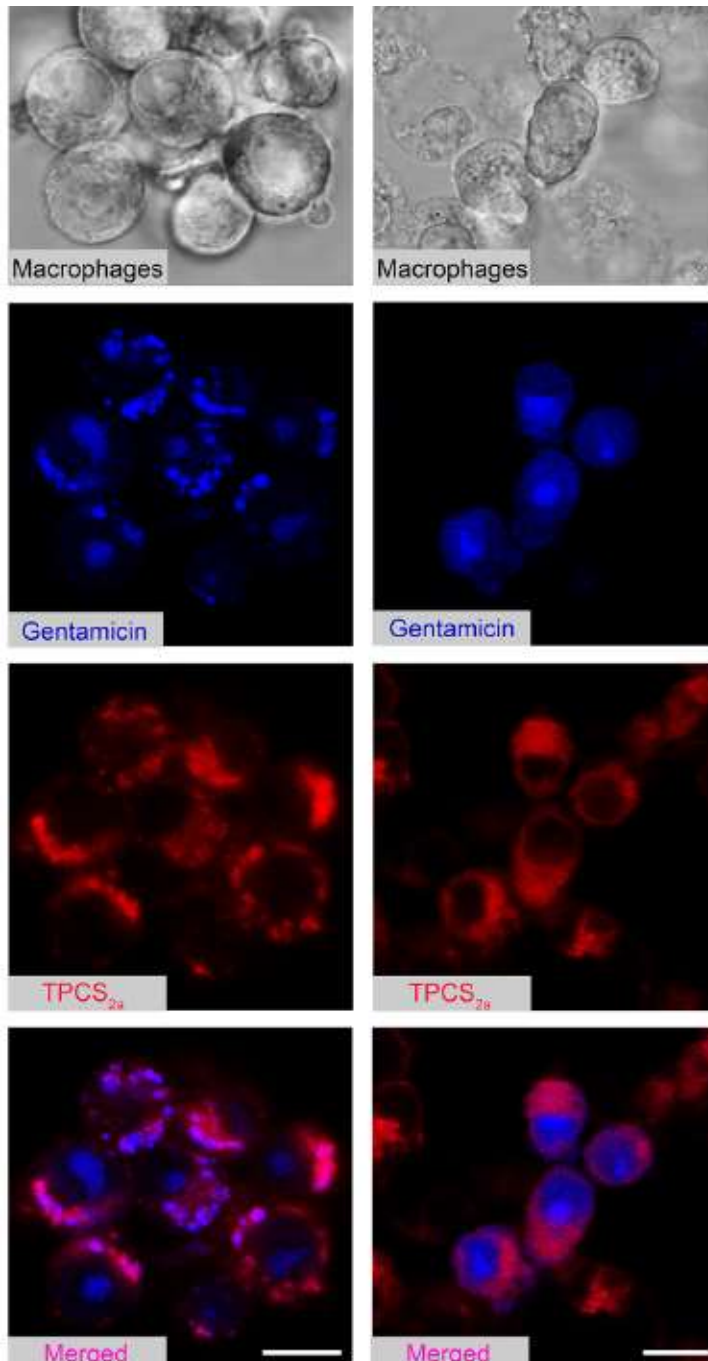


Mechanism of AM-PCI

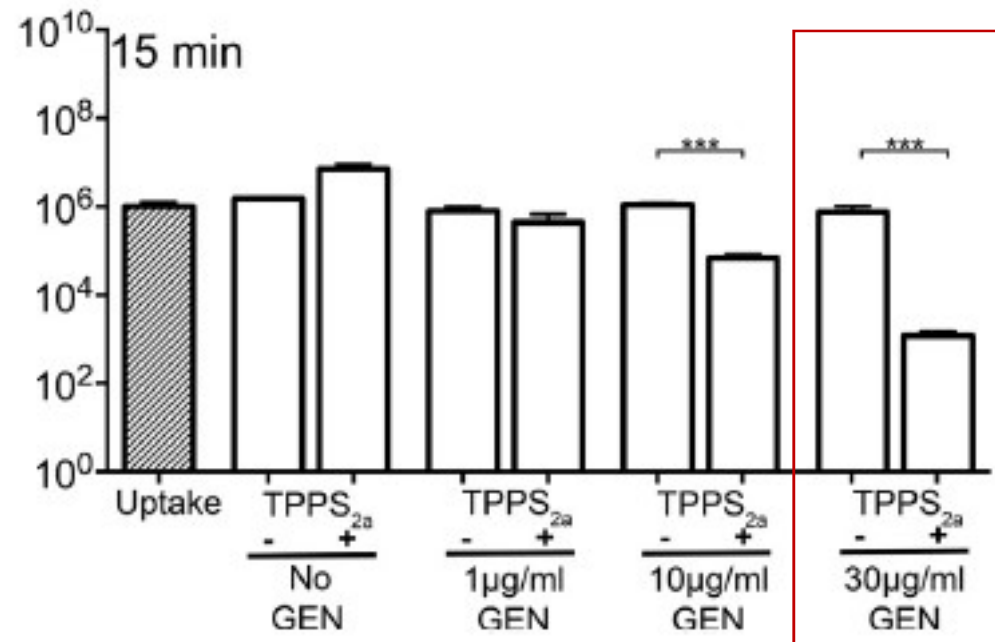




No illumination With illumination

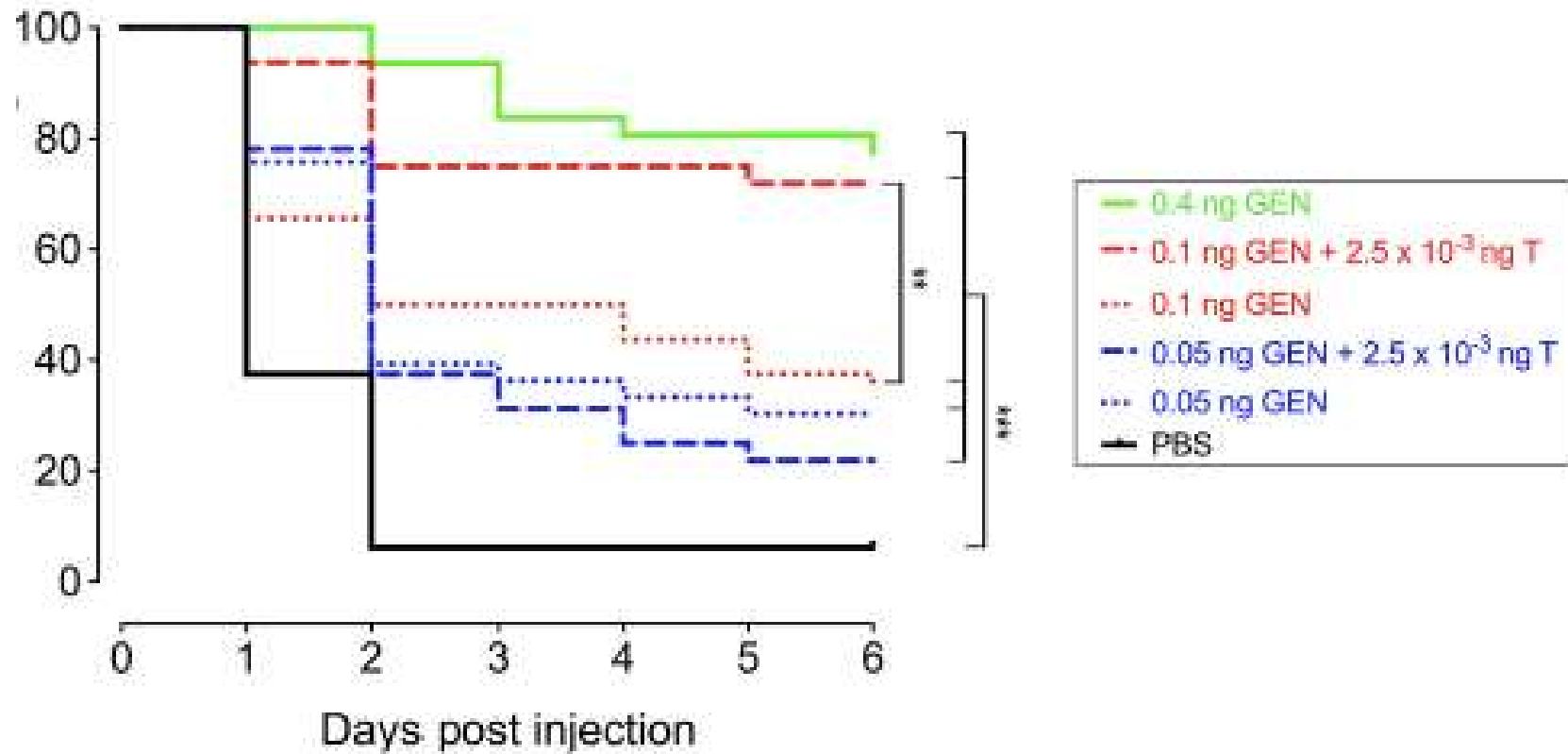


Photochemical internalization enhancement of gentamicin against intracellular *S. epidermidis*





Rescue of *S. aureus* - infected zebrafish embryos with gentamicin - PCI



- Non-toxic
- Effective protection owing to PCI



In summary: full circle!

- Platelets produce matrix shielding invading bacteria
- ES matrix also can provide shelter against immune cells
- Trombocidins protecting from NVE
- BALI novel SAAPs from LL-37 and Trombocidins
- SAAPs potent novel antimicrobials
- Self assembling polymers with AMPs
- Protect TE heart valves with SAAP- supramolecular system
- Kill intracellular bacteria with AM-PCI



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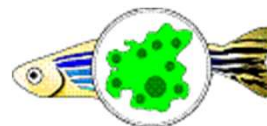
Anna Posthumus Meyes-de Breij
 Peter Nibbering
 Robert Cordfunke
 Pieter Hiemstra
 Jan-Wouter Drijfhout



Or Cohen
 Malka Reichart
 Noam Emanuel



Kristof Vercruyse
 Remko van Leeuwen
 Michel de Baar



ZF-screens



Moniek Schmitz
 Patricia Dankers



Nermina Malanovic
 Regina Leber
 Karl Lohner



Universiteit Leiden
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Platelet Microbicidal Activity Is an Important Defense Factor against Viridans Streptococcal Endocarditis

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Thrombocidins, Microbicidal Proteins from Human Blood Platelets, Are C-terminal Deletion Products of CXC Chemokines*

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Photochemical internalization enhances cytosolic release of antibiotic and increases its efficacy against staphylococcal infection

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